JOURNAL OF BACTERIOLOGY, Sept. 1979, p. 1049-1053
0021-9193/79/09-1049/05$02.00/0

Vol. 139, No. 3

NOTES

Small Arrays of Electron-Dense Cylinders in *Escherichia coli* Cells

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Received for publication 21 May 1979

Electron microscopy of unstained *Escherichia coli* cells from cultures kept near 0°C after incubation at 37°C revealed small areas of geometrically arranged electron-dense cylinders. Their morphology, organization, and occurrence are described.

The ultrastructural anatomy of *Escherichia coli* has been studied in detail for many years and it is unusual to find an undescribed feature. However, in the course of an examination of *E. coli* K-12 for other purposes, small areas of electron-dense spots in regular geometrically defined arrays were found in cells after storage in a refrigerator for a few days. They were present in several different strains, some carrying drug resistance plasmids and some not. The arrays were further examined as follows.

Various strains of *E. coli* and other bacterial species were used, details being included with results in Table 1. Cells were streaked on 2% agar plates (Mueller-Hinton agar or brain heart infusion agar; BBL Microbiology Systems) and incubated overnight at 37°C. Plates were stored at temperatures between −18 and +10°C for varying periods. Bacteria were suspended in drops of 0.1 M ammonium acetate solution (or water). A carbon-coated electron microscope specimen support grid was then touched onto the surface of the suspension, washed twice in 0.1 M ammonium acetate solution (or water), and dried. When negative staining was required, the grid was wetted with 0.2% sodium phosphotungstate solution before drying. Electron microscopy was carried out using a Philips EM 300 electron microscope with a goniometer stage (model PW6500/30) and a double condenser system. Whereas arrays were present in overnight Mueller-Hinton broth cultures and exponential-phase shake cultures which had been cooled to 0°C, relatively small numbers of cells from such cultures adhered to the carbon support films. Larger numbers of cells could be mounted from plate cultures, which were therefore preferred.

A typical array is shown in Fig. 1 (cut at the cell's midpoint), negative staining being used to outline the *E. coli* J53(N3) cell, although arrays were just as clear without it. They were not always diamond-shaped, being frequently rectangular (Fig. 2). Sometimes the spots merged and could not be easily distinguished (see Fig. 5). Each array consisted most frequently of about 36 spots (occasionally as few as 30 or as many as 80), which were 7 nm in diameter with a center-to-center spacing of 15 nm. Diamond-shaped arrays usually had six spots per side, giving a length of about 75 nm. Rectangles were more variable: 45 to 60 by 75 to 225 nm with the same spacing between the spots. In no case was more than one array visible in a cell; it was not confined to any particular region (e.g., the pole).

The spots were further studied using a goniometer (tilting) stage. Figure 3 shows that some of the spots in an array appear elongated. When the stage is tilted by 30° (Fig. 4) they become circular, indicating that the spots are in fact cylinders. They seem to be arranged in the same plane, but this has been distorted in Fig. 3 and 4 so that not all the rows of cylinders are in the same orientation with respect to the axis of tilt of the electron microscope stage. In Fig. 6, the cylinders are clearer; each array thus appears to consist of a number of "vertical" cylinders arranged in a "horizontal" plane. Their length (estimated by tilting) is about 25 nm. It is clear from Fig. 5 through 7 that the apparent disorganization of some arrays is due to orientation; as they are tilted, the arrays become regular.

In the course of studying bacteria which had been kept for long periods at 0°C (see below), it was found that many cells had disrupted, leaving empty cell envelopes, 5% of which carried arrays (usually disorganized). An intact and a partly

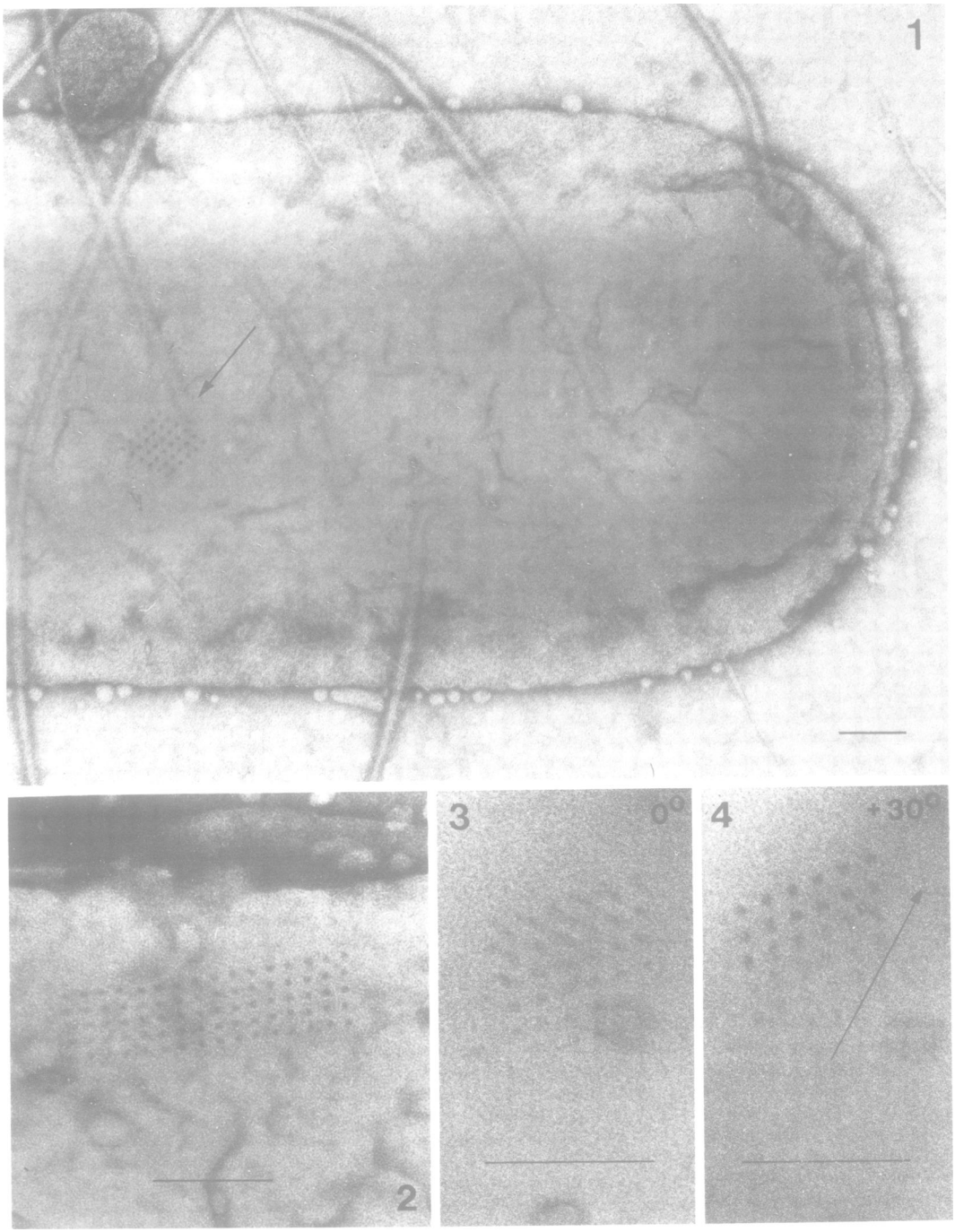

FIG. 1. *Cell of E. coli J53(N3) showing a diamond-shaped array (arrowed). The micrograph has been cut at the midpoint of the cell. Negatively stained; bar, 100 nm.*

FIG. 2. *Detail of a rectangular array at the edge of a cell of E. coli CR34-3. The array is not distorted by the wrinkles in the "upper" surface of the cell, so it must be either attached to the "lower" undistorted part of the cell envelope (flattened against the support film) or located intracellularly. Negatively stained; bar, 100 nm.*

FIG. 3. *Array in a cell of E. coli J53(N3) not tilted. Unstained; bar, 100 nm.*

FIG. 4. *The same as Fig. 3 tilted at 30° (axis of tilt arrowed). It is to be noted that some cylinders disappear on tilting due to contrast changes brought about by a reduction in the path length of the electron beam through them. Bar, 100 nm.*

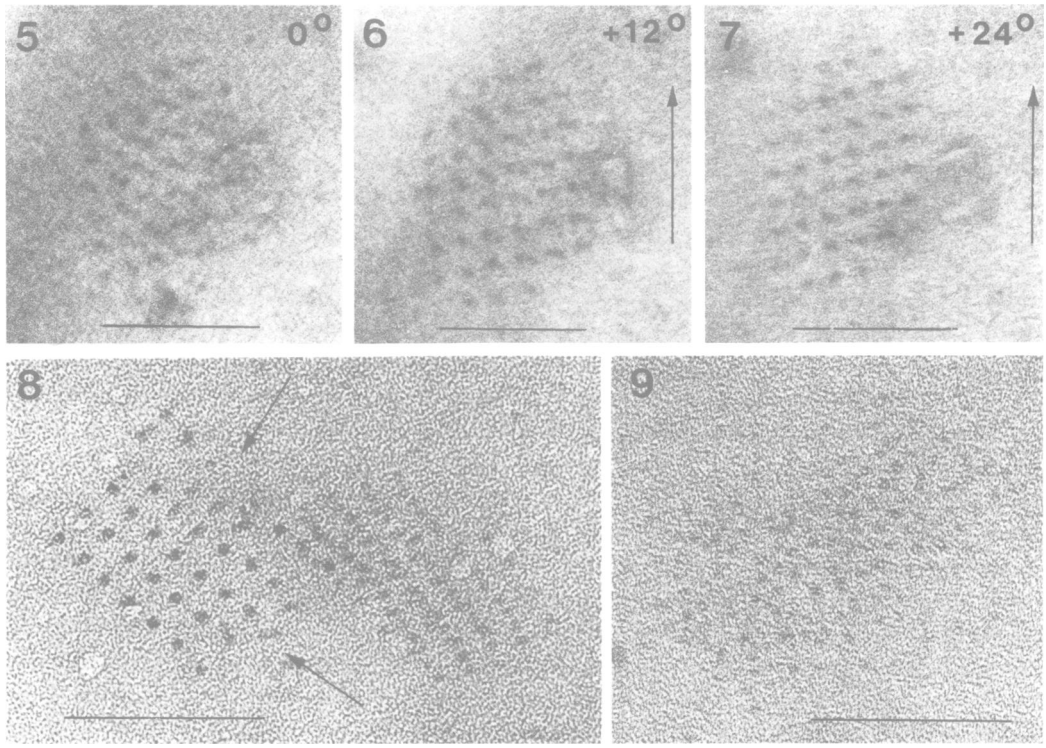

FIG. 5. *An apparently disorganized array in a cell of E. coli J53(N3) not tilted. Unstained; bar, 100 nm.*

FIG. 6 AND 7. *The same as Fig. 5 tilted at 12° and 24°, respectively (axes of tilt arrowed). The goniometer stage reduces resolution. Bar, 100 nm.*

FIG. 8. *Two arrays which were apparently in overlapping cell ghosts. Arrows mark tilted cylinders. The array on the right is partly disorganized. E. coli J53(N3) unstained; bar, 100 nm.*

FIG. 9. *Disorganized array in J53(N3) cell ghost. Bar, 100 nm.*

disorganized array in what appeared to be two overlapping cell ghosts are shown in Fig. 8. Tilted cylinders (arrowed) can be clearly seen in the former, whereas in the latter the spots are somewhat smaller, closer together, and no longer completely regular. This kind of disorganization, which is complete in Fig. 9, is probably not an orientation effect, but is most likely due to a structural breakdown of the cylinders. These observations do not indicate whether or not the arrays are attached to the cell envelope, but it seems unlikely that such defined periodic arrays could exist as free elements in the cytoplasm. Attachment to the organized molecular structure of one of the cell envelope membranes would seem more likely.

Arrays were first observed in *E. coli* K-12 strain CR34-3; they were more frequent in clones of strain J53, but were less frequent in strain C2 (see references 2, 3, and 8, respectively, for data on the strains). They were also numerous in a clinical isolate of *E. coli* (St. John's General Hospital). The presence of a drug resistance plasmid had no effect on their numbers. They were not present within the limits of detection in *Salmonella typhimurium* LT2, *Shigella sonnei*, or *Pseudomonas aeruginosa*, although their possible occurrence under different conditions cannot be ruled out. Table 1 summarizes these observations (*E. coli* CR34-3 is omitted since it does not grow on Mueller-Hinton agar).

The arrays are clearly not an artifact due, for example, to the specimen preparation procedure. If they were, one would expect to find similar structures in plate cultures not kept at low temperatures. In addition, more than one array would be present in each cell.

Arrays were not restricted to Mueller-Hinton agar, which was most suitable for their formation, but were much less frequent in cells grown on other media. For example, an overnight culture of *E. coli* J53(N3) on brain heart infusion agar gave 4% cells with arrays after 48 h at 3°C, and a similarly treated culture on M9 minimal medium (supplemented with glucose, proline, and methionine) had 12% of its cells with arrays.

TABLE 1. *Frequencies of regular arrays in bacterial strains*[a]

Strain	Source or reference	Cells with arrays (%)
E. coli		
K-12 J53(N3)[b]	N. Datta (3)	69
K-12 J53-1	N. Datta (3)	50
C2	Derivative of C (7)	0.05
B	ATCC 11303	0
Clinical isolate	P. Fardy	51
S. typhimurium LT2 SQ1139[c]	D. Bradley (2)	0
S. sonnei F3-2[c]	P. Fardy (2)	0
P. aeruginosa[c]	D. Bradley (5)	0

[a] A minimum of about 100 cells were screened for arrays from plate cultures (Mueller-Hinton agar), which had been stored at 3°C for 72 h, and mounted at room temperature for electron microscopy.

[b] Carries drug resistance plasmid N3 (4).

[c] No arrays could be found after varying periods at 3°C.

An overnight shake culture of *E. coli* J53(N3) in Mueller-Hinton broth gave 10% cells with arrays after cooling at 3°C for 5 h (the time to reach 3°C was about 1 h). Thus, array formation in broth appears to be as good as with plate cultures.

An attempt was made to ascertain the conditions required for the formation of arrays. *E. coli* J53(N3) was grown overnight at 37°C on Meuller-Hinton agar and examined after various temperature treatments. There were no arrays in bacteria examined immediately after removal from the incubator, or after standing for 2 h or more at room temperature (22°C), or after overnight incubation at 22°C. A period of time at low temperature seemed essential. The frequency of arrays in plate cultures stored for 24 h at various temperatures (0 to 10°C) is shown in Fig. 10. Their formation is maximal at 0°C, whereas none could be found at 10°C even after 110 h. It would seem likely that arrays are formed at temperatures below 0°C before the bacteria freeze (probably about −1°C). Quick-frozen cells (−18°C) did not have any arrays, but once formed, they were preserved at this temperature. Mounting on electron microscope specimen support grids was done at room temperature; processing in a cold room had no effect on the frequency. The rate at which arrays were formed was studied at 0°C (Fig. 11). Their initial formation was very rapid, but the rate of increase in frequency was reduced until a maximum of 82% was observed after 97 h (disrupted cells not scored). At this time, the culture was still viable. Although some cells were undoubtedly dead (disrupted ones), it seemed likely that many of the live cells carried arrays.

Arrays disappeared after reincubation. A plate culture with 28% of its cells with arrays was incubated for 2 h at 37°C; none remained after this treatment.

Apart from their regular arrangement, the most important features of these novel structures are their high density to electrons and association with low temperature; the latter could indicate that lipid-phase transitions are involved in their formation, but the former indicates the presence of a reasonably heavy element. This could be a metal chelated in the cold by some organic substance already existing as organized subunits. Clearly any consideration of the chemical nature of the arrays at this time is purely speculative. However, it is worth noting that the size of the rods and their spacing is very reminiscent of ferritin (7) and bacterioferritin (6). Iron is an essential element for bacteria, and in the absence of further data it seems possible that the arrays are one of a variety of iron-proteins known to exist bound to the cell mem-

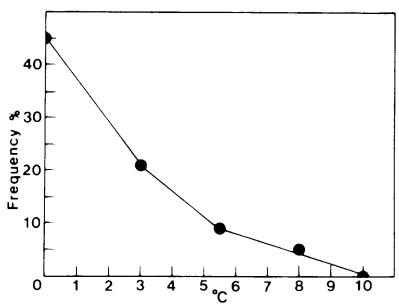

FIG. 10. *Relationship between temperature and the percentage of cells with arrays. Bacteria were held on plates at the indicated temperatures for 24 h and then scored for arrays. The strain used was E. coli J53(N3).*

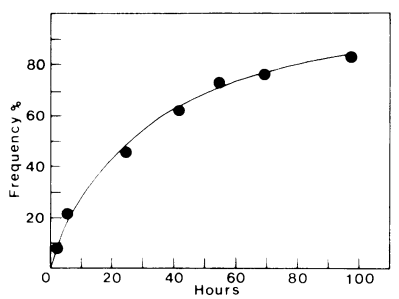

FIG. 11. *Rate of formation of arrays at 0°C. A Mueller-Hinton agar overnight culture of E. coli J53(N3) was held at 0°C (in ice) and sampled at the indicated times; cells with arrays were scored, empty ghosts not being counted.*

brane (and also in the cytoplasm), as mentioned by Bauminger et al. (1) in their study of iron in *E. coli* by Mössbauer spectroscopy.

I am grateful to those mentioned in Table 1 for supplying bacterial strains and to numerous colleagues for correspondence concerning these structures. Doris Cohen provided valuable technical assistance.

This work was funded by the Medical Research Council of Canada (grant MA5608).

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